Daily administration of m-chlorophenylpiperazine to healthy human volunteers rapidly attenuates many of its behavioral, hormonal, cardiovascular and temperature effects

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Abstract The serotonergic agent meta-chlorophenylpiperazine (m-CPP) increases temperature and plasma ACTH and other hormones and decreases social interaction, locomotor activity and food intake in rats, most likely via stimulation of 5-HT_2C receptors. Repeated daily administration of m-CPP to rats induces rapid tolerance to these effects of m-CPP. As m-CPP has been used in challenge tests and in preliminary treatment trials in humans, we evaluated the possible development of tolerance to m-CPP in ten healthy human volunteers using a double-blind, random assignment crossover study of placebo versus daily m-CPP infusions. Psychological and physical symptoms of anxiety, temperature, pupil size, diastolic blood pressure, and plasma ACTH, cortisol, and prolactin concentrations were increased by the first administration of m-CPP (0.08 mg/kg) compared to placebo. All of these responses were attenuated on m-CPP days 2 and 3. Plasma m-CPP levels did not differ across the 3 m-CPP days. Repeated m-CPP administration thus appears to induce rapid tolerance to its behavioral and physiological effects in humans. Further investigations of the mechanisms involved in the development of subsensitivity to m-CPP may contribute to increased understanding of the regulation of serotonin-mediated functions and of anxiety disorders.

Key words Serotonin · m-CPP · Anxiety · Tolerance · Humans · ACTH · Prolactin · Cortisol · Mydriasis · Body temperature · Blood pressure

Introduction

Meta-chlorophenylpiperazine (m-CPP) is a serotonergic agent which elicits changes in many behaviors and physiological functions in rodents, non-human primates and humans (reviews: Kahn and Wetzler 1991; Murphy et al. 1991; Kennett 1993). In humans, there has been particular interest in m-CPP as a probe of central serotonin (5-HT) systems which may modulate anxiety. Acute administration of m-CPP produces psychological and physiological manifestations of anxiety in neuropsychiatric patients and healthy volunteers. In animal studies using partially selective serotonin antagonists and other antagonists, and cross-tolerance investigations using 5-HT_1A, 5-HT_2A and 5-HT_2C agonists, the effects of m-CPP on temperature, plasma prolactin and other hormones, food intake and anxiety-like behaviors have been attributed to actions mediated by post-synaptic 5-HT_2C receptors, at which sites m-CPP is a partial agonist (Conn and Sanders-Bush 1987; Kennett et al. 1989, 1994b; Murphy et al. 1991; Aulakh et al. 1992a; Calogero et al. 1993; Kennett 1993; Mazzola-Pomietto et al. 1996; Callahan and Cunningham 1994). Further supporting this view, initial studies of transgenic mice lacking 5-HT_2C receptors have shown a complete absence of the usual food intake reduction produced by m-CPP (Tecott et al. 1995).

In rats, several effects of m-CPP are attenuated or extinguished following repeated, daily m-CPP administration. m-CPP-induced reductions in food intake or increases in temperature or ACTH were essentially completely eliminated by 2–5 days of repeated m-CPP administration (Aulakh et al. 1994a, 1995; Mazzola-Pomietto et al. 1994). In other investigations, m-CPP administration for 14 days led to attenuation of m-CPP-induced reductions in locomotor activity, social interaction and food intake; intermediate time points were not examined in these studies (Sills et al. 1985; Kennedy et al. 1993). In electrophysiological studies,
cultured A9 cells transfected with 5-HT_{2C} receptors manifested attenuated responses to 5-HT after long-term (24 h) but not short-term (1 h) incubation with m-CPP (Boddeke et al. 1993).

In humans, m-CPP has been re-administered in both oral and intravenous single-day challenge tests after intervals of 10 days to 2 months without any apparent reported alterations in endocrine or other responses, although this was not a specific focus of these studies (Zohar et al. 1988; Hollander et al. 1991; Owen et al. 1993; Jacobsen et al. 1994; Schwartz et al. 1995). In two different studies, daily administration of m-CPP for 2 weeks to neuropsychiatric patients led to modest changes in depression ratings (Mellow et al. 1990; Lawlor et al. 1991). A preliminary report of a 10-week trial of m-CPP in obsessive-compulsive patients indicated some therapeutic benefit (Pigott et al. 1992). These pilot therapeutic trials were undertaken with the hypothesis that repeated administration of m-CPP might induce down-regulation or desensitization of serotonergic function, possibly including 5-HT_{2C} receptor functions, but specific indices of such changes were not measured.

In studies using other serotonergic agents in humans, plasma prolactin responses to fenfluramine were essentially identical when measured after an interval of 1 week (Stoff et al. 1992). Diminished prolactin responses, however, were observed following a second fenfluramine challenge test administered 2–12 days after the first test in another study (Coccaro et al. 1987). Plasma prolactin responses to intravenously administered clomipramine were attenuated 2 weeks, but not 4 weeks, after a single administration of clomipramine (Gilmore et al. 1993). Both fenfluramine and clomipramine act presynaptically, fenfluramine primarily as a 5-HT releasing agent and clomipramine as a 5-HT uptake inhibitor. Thus, possible tolerance development to their administration could reflect either presynaptic changes or post-synaptic adaptation at a number of serotonin receptors, as well as changes specific to prolactin release, since other dependent variables were not examined in these studies.

The present study was undertaken to evaluate whether the daily administration of m-CPP to healthy human volunteers might lead to the development of tolerance to its behavioral or physiological effects. We therefore designed a double-blind, random assignment, crossover study using repeated doses of intravenous m-CPP versus placebo and examined the following dependent variables: self-reported ratings of anxiety and other mood states, temperature, blood pressure, heart rate, pupil size, plasma hormones, and plasma m-CPP concentrations. We also sought to evaluate whether a 20% reduction in dose of m-CPP (0.08 mg/kg) might be reliably distinguished from placebo. While an m-CPP dose of 0.1 mg/kg has been generally well tolerated using a 90-s intravenous infusion paradigm (Murphy et al. 1991; Pigott et al. 1993), with this lower dose we hoped to avoid or minimize effects reported to be elicited at non-serotonin receptor sites by relatively high m-CPP concentrations used in some studies in animals and in vitro (Hamik and Peroutka 1989; Murphy et al. 1991; Wolf and Kuhn 1991; Baumann et al. 1993; Carver and Grahame-Smith 1993; Hoyer et al. 1994).

**Materials and methods**

**Subjects**

Ten healthy adult volunteers were studied, six men and four women; mean age was 32.4 ± 3 years. The volunteers underwent a clinical screening and examination with the overview section of the SCID questionnaire (Spitzer et al. 1990) to exclude mental disorders. They also underwent a medical interview, physical examination and routine laboratory tests to exclude medical illness, pregnancy and psychotropic drug use. All subjects gave written informed consent.

**Design**

Each subject received m-CPP infusions in identical doses for 3 days in a row; on the day before or the day after this series of three challenges, the volunteer received a placebo infusion. The order (placebo first or last) was randomly assigned and double-blind, i.e., placebo, m-CPP, m-CPP, m-CPP; or m-CPP, m-CPP, placebo. Six subjects received placebo before the repeated m-CPP challenges, and four subjects received it after them. Subjects received the following explanation: "The purpose of this study is to evaluate your response to repeated doses of a drug, meta-chlorophenylpiperazine (m-CPP). We will compare your responses to m-CPP with your responses to placebo, a dummy substance like sugar, in order to assess the effects of simply participating in the study separately from the effects of the drug."

**Procedures**

On challenge days, subjects fasted with only water permitted from midnight and throughout the challenge. At about 0900 hour, two intravenous lines were inserted and the subject allowed to acclimatize while reclining in a chair or bed; sleep was not permitted. At 1000 hour (time "0") subjects received m-CPP or placebo through one intravenous line, which was then removed. Subjects received m-CPP (0.08 mg/kg body weight) in 20 ml saline administered over 90 s on three successive mornings (Murphy et al. 1989); placebo was administered under identical conditions on day 1 or 4 as described above. m-CPP hydrochloride was purchased from Aldrich Chemical Co., Milwaukee, Wisc. The identity and purity of the m-CPP was verified by the NIH Clinical Center Pharmaceutical Development Service, Bethesda, Md.

**Assessments**

Blood pressure, heart rate, estimated pupil size in mm, and oral temperature were monitored every 15 min throughout the studies. Before the infusion, at −30 min and 0 min, and after the infusion, at +30, +60 and +90 min, 8.5 ml blood were drawn through the second intravenous line for measurement of cortisol, adrenocorticotropic hormone (ACTH), prolactin and m-CPP. Blood was drawn into tubes containing EDTA, kept on ice until +90 min, and...